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Terconazole Proniosomal Gels: Effect of Different Formulation Factors, Physicochemical and Microbiological Evaluation

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Abstract

As treatment of vaginal fungal infection depends mainly on slow release of the drug and prolonged contact time of the delivery system with the vaginal mucosa, proniosomal gel was used as promising candidate to achieve this target. Terconazole, antifungal drug, proniosomal gels were developed based on span 60 and Brij 76 in different molar ratios (1:1, 1:1.5 and 1:2) relative to cholesterol. Proniosomal formulations were hydrated to form niosomes by incorporating into 1% carbopol gel. Proniosomal gel formulations were evaluated for their Entrapment Efficiency (EE%) and vesicle size. Increasing the molar ratio of cholesterol relative to surfactant has affected both EE and vesicle size of prepared niosomes. Drug release profile from different prepared proniosomal gel formulations in simulated vaginal fluid (SVF) was studied in comparison to the commercial product of terconazole for 24 hours. Depending on the high EE% and in-vitro release profile of formulation SC1.5 (1:1.5 span60: cholesterol), it was selected for further evaluations of stability, mucoadhesion to the vaginal mucosa and inhibition of candidas growth. Results indicated that the selected formula, SC1.5, showed good stability and provided higher mucoadhesion and retention time then the commercial product which resulted in more efficient in-vitro inhibition of candida albicans.

Keywords

Proniosomes; Terconazole; Cholesterol; Span; Brij; Carbopol; Mucoadhesion

Introduction

Drug delivery systems using colloidal particulate carriers such as liposomes or niosomes have distinct advantages over conventional dosage forms. These carriers can act as drug reservoirs, and modification of their composition or surface can adjust the drug release rate and/or the affinity for the target site [1]. However, there remain significant problems in the general applications of liposomes for drug delivery. In a dispersed aqueous system, liposomes have problems associated with degradation by hydrolysis or oxidation [2] as well as sedimentation, aggregation, or fusion of liposomes during storage [3]. Other problems associated with clinical applications of liposomes include difficulties in sterilization and in large-scale production to obtain a product with adequate physical and chemical stability [4]. Proniosomes have generated interest as a topical formulation as an approach to avoid the side effects associated with oral administration. Proniosomes offer a versatile vesicle drug delivery concept as they give niosomes after being hydrated either before application or by the effect of skin or mucosal hydration with minimized problems of physical stability [5]. Formation of the pronisomal gel depend on use of many components, among the most important structural components, Non-ionic surfactants are used. They act as vesicle forming agents, the nature of vesicles formed depends upon HLB value in addition to phase transition temperature. Another important component is cholesterol which acts as "vesicular cement" in the molecular space that formed by the aggregation of monomer to form the bilayer [6]. On the other hand, incorporation of lecithin into the proniosomal formulation acts as stabilizing agent [7]. Vaginitis is one of the most frequent genital infections occurring in women of all age groups [8]. It has been reported that 30–35% of vaginitis episodes are due to Candida albicans. About 75% of women experience an acute episode of vaginal candidiasis once in their lifetime, most commonly during pregnancy or after treatment with antibiotics [9]. So, increased incidence of vaginal candida requires efficient medication which provides sustained localized release of the antifungal agent avoiding the toxic effect after oral administration of antifungal drugs [10,11]. The use of bioadhesive polymers can enhance the sustained and localized vaginal delivery of the drug as it acts to prolong the contact time between the dosage form and the mucosa which retains drugs for treating largely local conditions. Antifungal imidazole drugs are a main stay in the treatment of fungal infections. Imidazole drugs have low aqueous solubility because of their hydrophobic structures. Terconazole 1-[4-[[(2RS,4SR)-2-(2,4-dichlorophenyl)-2-[(1H-1,2,4-triazol-1-yl)methyl]-1,3-dioxolan-4yl] methoxy] phenyl]-4-(1-methylethyl)piperazine is a triazole antifungal agent which shows greater efficiency, shorter treatment regimens, lower relapse rates and better mycologic and clinical cure rates in comparison with other imidazole antifungal agents in the treatment of vulvovaginal candidiasis [12]. The aim of this study was to formulate and evaluate proniosomal gel formulations of antifungal drug to enhance treatment of vaginitis through controlled drug release and prolonged contact time.

Materials

Terconazole nitrate was donated from Amriya Pharmaceuticals Ind. (Alexandria, Egypt). Span 60 and Brij 76 were supplied from Koch-light laboratories Ltd (Colebrook Bucks, England). Lecithin was obtained from Merk Company (Darmstadt, Germany). Cholesterol, acetic acid and ethanol were supplied from Riedel Dehae¨n (Darmstadt, Germany). Acetonitrile HPLC grade, potassium dihydrogen phosphate, and disodium hydrogen phosphate were purchased from BDH Laboratory Supplies (BDH Chemicals Ltd, Poole, UK). Carbopol 934 was supplied by Luna Co. Goodrich, USA).

Methods

Preparation of simulated vaginal fluid (svf)

The SVF was prepared from 3.51 g/l NaCl, 1.40 g/l KOH, 0.222 g/l Ca(OH)2, 0.018 g/l bovine serum albumin, 2 g/l Lactic acid, 1 g/l acetic acid, 0.16 g/l glycerol, 0.4 g/l urea and 5 g/l glucose. The pH of

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the mixture adjusted to 4.5 ± 0.2 using 0.1 N HCl [13,14]. Terconazole solubility in the prepared SVF was determined by vial and shaker method and it was found to be 0.21 ± 0.03 mg/ml. Then, terconazole calibration curve was constructed.

Preparation of terconazole proniosomes

Proniosomes were prepared using a method mentioned by Ibrahim [1]. The compositions of different proniosomal formulations are listed in Table 1. Terconazole was used as 1.6 %w/w in each formula. Two surfactants, span 60 and brij 76, were used in three different molar ratios, 1:1, 1:1.5, and 1:2 relative to cholesterol ratio. Constant amount of lecithin in one molar ratio was used in each formula. Terconazole along with surfactant, lecithin, and cholesterol was mixed in wide-mouth glass tube with 10 ml of absolute ethanol. Then the open end of the glass tube was covered with a lid and the tube was warmed in a water bath at 65 ± 3 °C for 5 min. Then 1.6 ml of pH 7.4 phosphate buffer was added and the mixture was further warmed in the water bath for about 2 min so that a clear solution was obtained and then was allowed to cool to room temperature.

Preparation of Terconazole proniosomal gel

Carbopol gel 1% w/v was prepared by soaking carbopol 934 (1g) in 100 ml distilled water for 3 hours, the mixture was transferred to mechanical stirrer at 100 rpm to form homogenous viscous solution. Few drops of triethanolamine (5-7 drops) were added until formation of the gel. As the concentration of terconazole in all proniosomes preparation is 1.6% (1.6 mg/100 mg), one gm of the prepared proniosomes is mixed with one gram carbopol gel to give final concentration of terconazole of 0.8% in the prepared proniosomal gels.

Evaluation of the Prepared Terconazole Proniosomal Gels

Encapsulation efficiency (EE%)

Weighed amount, 0.5 g, of each formula was mixed with 10ml SVF pH 4.5 in glass tube, the aqueous suspension was sonicated for 10 min and then exposed to centrifugation at 25,000 rpm for 30 min to separate the terconazole niosomes. The supernatant was recovered and assayed by spectrophotometric UV analysis (Schimadzu spectrophotometer. Model UV-1601, Japan) at λ_{max} 227 nm. The percentage of drug encapsulation efficiency (EE%)was calculated as follow:

EE%= total terconazole - terconazole in the supernatant × 100.

Vesicle physical analysis

Scanning electron microscope (TEM; JEOL, Tokyo, Japan) was used for observation of the shape and size of terconazole niosomes. Proniosomal gel was diluted with 10 ml of SVF pH 4.5, the resulted niosomes were mounted on copper grid coated with carbon film and scanned for its shape and size. Experiment was done three times and average vesicle size was calculated.

In-vitro release study

The dissolution test of different prepared terconazole proniosomal gels was done using USP dissolution tester, apparatus II (Hanson Research, SR8 plus, Dissolution test Station, USA) The USP dissolution apparatus was modified with a "small volume" adapter kit (p/n 72-800-721, Hanson Research), including 150 ml flasks and mini paddles. One gram of the proniosomal gel as well as the commercial cream was spread on glass circular disk (2.5cm diameter), then covered by cellophane dialyzing membrane with molecular weight cut-off of 500-1000Da (Spectrum Medical Inc., Los Angeles, CA, USA) which was securely mounted on the disk by a rubber band. The disk was placed on the bottom of the dissolution tester vessel and 100 ml of simulated vaginal fluid (SVF) pH 4.5 was poured carefully on the wall of the vessel. The temperature was maintained at 37 ± 1 ° C with paddle speed rotation of 50 rpm. For each formula, drug release was studied in triplicate. At predetermined time intervals up to 24 hours, 2 ml aliquots of the dissolution medium were withdrawn and immediately replaced by an equal volume of SVF pH 4.5 pre-adjusted to 37 ^{0}C to maintain sink conditions. The samples were analyzed by a validated spectrophotometric method at λ_{max}= 227 nm and average percent of drug release at each time interval was calculated.

Stability studies

The selected formula SC1.5 was stored at 40 ^{0}C, 75% RH for three months. At the end of this period, it was analyzed for its EE % and drug release. Results were compared with those of freshly prepared ones.

Ex-vivo evaluation of the selected Terconazole proniosomal gel formula

Mucoadhesion and retention measurement in SVF

The mucoadhesion and retention measurement of the selected formula SC1.5 compared with the commercial cream of terconazole (Terazol®3) in SVF was assessed using fabricated system (Figure 1) [15,16] to measure the amount of drug retained on the mucosa against time. Isolated sheep vaginal mucosa was obtained from local slaughterhouse immediately after the animal was killed, deprise and supporting tissues were removed and the mucosa was kept in SVF in freezer until use. Sheep vaginal mucosa was cut into 5-cm×5-cm pieces and mounted on simulated vaginal system with mucosal side up. The proniosomal gel formula and the commercial cream, 1gm each, were mounted on the mucosal membrane on determined area (1 cm x 2 cm), and SVF was applied on both using polypropylene bag through an intravenous infusion set at a constant flow rate of 5 mL/h. At pre-determined time intervals, dispersion was collected into a receiver beaker, which was analyzed spectrophotometrically and the percentage drug retained on vaginal mucosa was calculated.

In-vitro inhibition of Candidas (yeast)

In vitro inhibition of candidas (yeast) by terconazole proniosomal

Table 1: Composition of different terconazole proniosomal gel formulations.

Molar ratio of surfactant: cholesterol	1: 1		1:1.5		1:2	
Formula	SC1	BC1	SC1.5	BC1.5	SC2	BC2
Span 60 (HLB 4.7)* (MW:430 g/mole)	43 mg	-------	43 mg	------	43 mg	
Brij 76 (HLB 12.4)* (MW: 712g/mole)	------	71.2 mg	-------	71.2 mg	---------	71.2 mg
Cholesterol (MW: 338g/mole)	38.8 mg	38.8 mg	58.2 mg	58.2 mg	77.6 mg	77.6 mg
Lecithin (MW: 800 mg)	8 mg	8 mg	8 mg	8 mg	8 mg	8 mg
Phosphate buffer	1.6ml	1.6ml	1.6ml	1.6ml	1.6ml	1.6ml
Terconazole (1.6%)	27.04 mg	27.49 mg	27.35 mg	27.80 mg	27.66 mg	28.11 mg

*HLB: Hydrophilic Lipophilic Balance

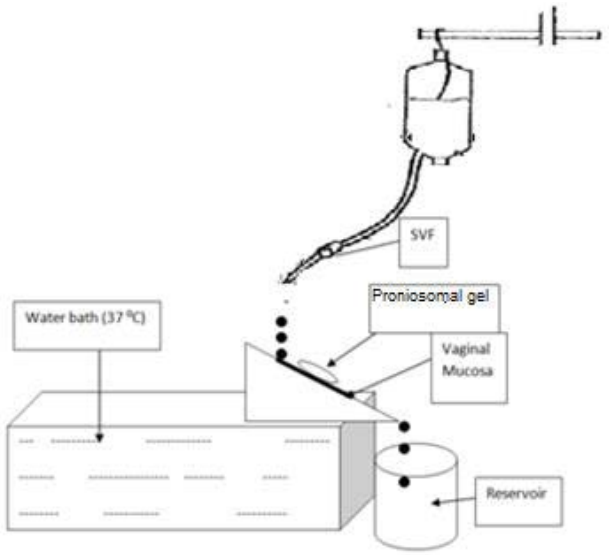

Figure 1: Fabricated system for measurement of mucoadhesion and retention time.

formulation (SC1.5) and commercial cream (Terazol'3) was estimated by using cup plate method [17,18]. In addition to formula SC1.5 and the commercial cream, withdrawn dissolution samples of formula SC1.5 in SVF at different time intervals were tested for its inhibition of candida growth. After autoclaving, first base agar (3% w/v) was poured into sterile petri dish (15 cm in diameter) and allowed to solidify. Five milliliter of standardized suspension of yeast (150cells/ml) was uniformly mixed with 50 ml of 1% w/v MRS agar (top agar) and then plated on previously solidified base agar plate. Wells were made in the plate using an 8-mm borer. Test samples (0.5 ml for dissolution samples and 0.25 gm for proniosomal gel and commercial cream) were poured into the wells, and plates were incubated at 37 ± 2°C for 24 h. Zone of inhibition (ZOI) of different samples was measured using calipers in terms of its diameter. Each sample was tested three times and average ZOI was calculated.

Statistical analysis

Data, expressed as the mean of three experiments, and the standard deviation (SD) were analyzed using one way analysis of variance (ANOVA). Statistical differences yielding $P<0.05$ were considered significant.

Results and Discussion

Entrapment efficiency

The entrapment efficiency is one of the most important parameter from pharmaceutical viewpoint in niosomal formulations. Entrapment efficiencies of different prepared formulations are collected in Table 2. It was observed that all the prepared formulations could entrap terconazole and form vesicles as terconazole is hydrophobic drug and it tends to be housed within the lipid bilayer of niosomes formed of surfactants and cholesterol.

Effect of surfactant type on EE%

In general, except for SC2 and BC2, it was observed that EE% of formulations prepared by span 60 is higher than those prepared with brij76 ($p>0.05$) at the same molar ratio of surfactant relative to cholesterol. From Table 2, Formulations SC1 and BC1 have EE% of 83.2 ± 3.8 and 48.3 ± 5.2 respectively while formulations SC1.5 and BC1.5 have EE% of 94.8 ± 2.7 and 72.3 ± 4.1respectively. It was reported previously that the HLB number lies between 4 and 8 is more compatible with vesicle formation and also higher T°C values are more likely in the ordered gel form forming less leaky bilayer, thus having higher entrapment efficiency [19,20]. The HLB values for span 60 and brij 76 are 4.7 and 12.4 respectively and their T°C values are 50°C and 30 °C respectively, which explains why niosomes formed by span 60 have higher EE% than those formed with brig 76 at the same molar ratios. An exception from the above conclusion, formula BC2 has EE% of 84.5 ± 4.3 which is significantly higher than those obtained by span formula at the same molar ratio, SC2, which has EE% of 75.5 ± 3.6, this is related to incorporation of high amounts of cholesterol relative to span (1:2 span: cholesterol) which may compete with the drug for packing space within the bilayer, hence excluding the drug as the amphiphiles assemble into the vesicles [21].

Effect of cholesterol ratio on the entrapment efficiency

Formulations prepared with span 60 in different molar ratio of cholesterol (SC1, SC1.5, and SC2), increasing the molar ratio of cholesterol from 1 to 1.5 relative to span 60 have significant ($P<0.05$) increase of the EE% from 83.2 ± 3.8 to 94.8 ± 2.7 which may be related to that span 60 has long saturated alkyl chain, so, with increased cholesterol, the bilayer hydrophobicity and stability increased [22] and permeability decreased [23] which may lead to efficiently trapping the hydrophobic drug into the bilayers as vesicles formed. On the other hand, further increase of the molar ratio of cholesterol from 1.5 to 2 relative to span 60 have significantly ($P<0.05$) decreased the EE% from 94.8 ± 2.7 to 75.5 ± 3.6 as higher amounts of cholesterol may compete with the drug for packing space within the bilayer [21], as mentioned above, and thus decreasing the EE%. Formulations prepared with brij76 (BC1, BC1.5, and BC2) with different molar ratio of cholesterol have showed significant ($P<0.05$) increase in the EE% with increase in the molar ratio of cholesterol from 1 to 1.5 and finally to 2. This may be explained in that as the HLB of the surfactant increases above 10, (for brij76, it is 12.5), the minimum amount of cholesterol necessary to form vesicles increases [24], high HLB value of 12.6 indicates low hydrocarbon chain volume in comparison with hydrophilic surface area. Thus, increased cholesterol content might have increased the lipophilic behavior of the lipid bilayer of Brij76 niosomes and crystallinity of the bilayer [24] resulting in increasing the EE%.

Vesicle physical analysis

The scanning electron microscopy images of the niosomes prepared from different terconazole proniosomal formulations are shown in Figure 2 and their vesicle mean size is represented in Table 2. Vesicles of all formulations were spherical and discrete with sharp boundaries.

Table 2: Physicochemical characterization of terconazole proniosomal formulations.

Formulations	EE %	Vesicle mean size (μ m)
SC1	83.2 ± 3.8	13.2 ± 1.2
SC1.5	94.8 ± 2.7	9.8 ± 1.6
SC2	75.5 ± 3.6	4.6 ± .8
BC1	48.3 ± 5.2	15.4 ± 0.9
BC1.5	72.3 ± 4.1	12.4 ± 1.3
BC2	84.5 ± 4.3	8.3 ± 1.1

Each value represents the mean ± SD (n=3)

Effect of surfactant type on vesicle size

It was observed that niosomes prepared with Brij76 were significantly larger (p>0.05) than those prepared with Span 60 which may be attributed to that the higher hydrophobicity of span 60 over Brih 76.

Effect of cholesterol ratio on vesicle size

From the results, there is inverse relationship between vesicle mean size and amount of cholesterol used as in all formulations. Higher amounts of cholesterol increase the vesicle hydrophobicity and decrease its surface energy which results in decrease in vesicle mean size.

In-vitro release study

Terconazole release from different prepared proniosomal gels compared to the commercial product of terconazole, Terazol®3, is shown in Figure 3 and amount remained after different time intervals is represented in Figure 4. It can be noted that all formulations have initial drug release (up to 1hour) which mostly depended mainly on the free un-entrapped drug and irreversibly related to the EE%. As a result, Terazol®3 has the highest percent of drug release up to 1 hour. Following this time, niosomes, resulted from hydration of proniosomes with the dissolution medium, release terconazole depending mainly on their structure.

Effect of surfactant type and cholesterol ratio on drug release

Formulations prepared by Brij 76 in the two molar ratios 1:1 and 1:1.5 have higher drug release than those prepared by span 60 at the same molar ratio (P<0.05) which is related to the less hydrophobicity of brij 76 than span 60. On the other hand, formula BC2, with double molar ratio of cholesterol relative to brij 76, has much slower drug release than SC2 (p= 0.0001) which is prepared at the same molar ratio, as the amount of cholesterol in BC2 introduces highly hydrophobic environment that the drug cannot escape to the aqueous dissolution medium. It was mentioned by Vemuri and Virtanen [25,26] that increasing the cholesterol content relative to the surfactant results in a more intact lipid bilayer as a barrier for drug release and decreased its leakage by improving fluidity of the bilayer membrane and reducing its permeability, which leads to lower drug elution from the vesicles. The same ratio of cholesterol was used in formula SC2 which has drug release significantly higher than BC2 and much close to that of the commercial cream, this phenomena was explained previously by EL-Samaligy [27] and New [28], who reported that increasing cholesterol beyond a certain concentration, in presence of certain type of surfactant, can disrupt the regular linear structure of the vesicular membrane and increase the drug release. Formulations SC1 and BC1 have released more than 95% of the drug after 9 and 3 hours respectively with significant difference between each other (p-value = 0.0205). Comparing them with the release profile of commercial product Terazol®3 resulted in significant difference with SC1 (p-value = 0.0307) and not quite statistically significant difference with BC1 (p- value = 0.0662). Statistical comparison of the release profile of the two formulations prepared by molar ratio 1:1.5, SC1.5 and BC1.5, resulted in that formulation SC1.5 has slower drug release than BC1.5 (P= 0.0066) which extended up to 24 hours which can be useful in-vivo to give continuous drug release all-over the day. It is worthy to mention that formula SC1.5 has the highest EE% among the prepared formulations which indicates that the release profile of this formulation depends mainly on presence of the drug into the formed niosomes whose structure resulted in this optimized release profile. Depending on the high EE% and optimized release profile, formulation SC1.5 was selected for further evaluation tests.

Stability studies

After the selected formulation SC1.5 was subjected to storage under specific conditions (40°C 75%RH), the EE% was determined and was found (94.4 ± 2.6%) with non-significant difference (P=0.7955) between EE% of the freshly prepared formulation (94.8 ± 2.7%). The release profile of stored SC1.5 was non-significantly different from

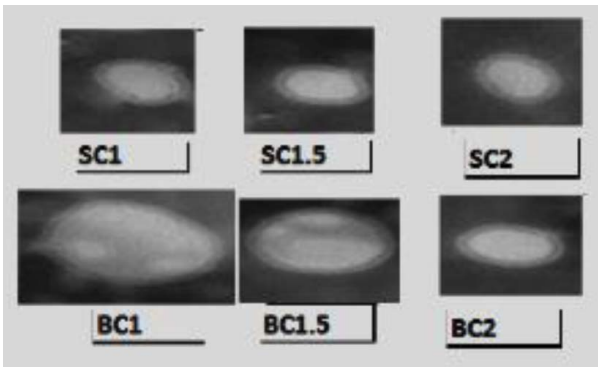

Figure 2. Scanned electron microscopy (SEM) images of different terconazole proniosomal formulation

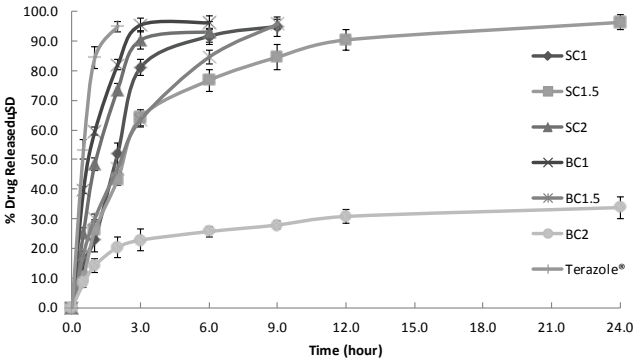

Figure 3: Terconazole release from its prepared proniosomal gels in SVF.

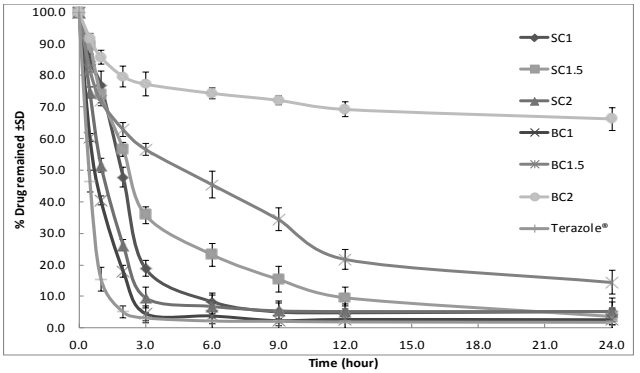

Figure 4: Terconazole remained in its proniosomal gel formulations after different time intervals in SVF

the fresh one (P=0.9733) as the fresh and stored formulations released 96.4 ± 2.6 and 95.7 ± 1.9 % of terconazole, respectively, after 24 hours.

Ex-Vivo Evaluation of the Selected Terconazole Proniosomal Gel Formula (SC1.5)

Bioadhesion and retention measurement in SVF

Bioadhesion may be defined as the state in which two materials, at least one of which is biological in nature, are held together for extended periods of time by interfacial forces. In the pharmaceutical sciences, when the adhesive attachment is to mucus or a mucous membrane, the phenomenon is referred to as mucoadhesion [29]. Figure 5 shows the relationship between time and amount of terconazole remained on the vaginal mucosa calculated from the collected amount in the SVF as a result of proniosomal gel de-attachment from the vaginal mucosa of both formula SC1.5 and commercial product Terazol®3. It can be said that the bioadhesivness of the prepared proniosomal gel is significantly higher than the commercial product which has completely removed from the mucosa after 1 hour while formula SC1.5 has been completely removed after 3 hours due to presence of the bioadhesive polymer carbopol 934 which forms strong interaction with the vaginal mucus lining the tissue [29] and thus increases the contact time and permits controlling the drug release.

In-vitro inhibition of Candidas (yeast)

Samples from formula SC1.5 dissolution test for terconazole release were tested for their inhibition of yeast growth on agar media, results of inhibition zone are shown in Table 3. It is clear that increase in drug release with time resulted in increase in the inhibition zone value. By representing the inhibition zone values against the cumulative drug release in SVF at the same time intervals, Figure 6, it was found that there is excellent correlation between the represented data (R2=0.9918). On the other hand, there was significant difference between inhibition zone of the proniosomal gel SC1.5 and the commercial product (Terazol® 3) (P<0.05) which gives indication that the formulated proniosomal gel enhanced terconazole release and diffusion into the agar media thus elevated its activity against yeast growth.

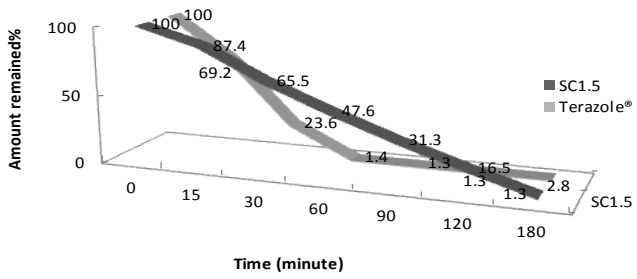

Figure 5: Terconazole amount remained on the vaginal mucosa of the selected formula SC1.5 relative to the commercial product.

Table 3: Inhibition zone of SC1.5 dissolution samples, SC1.5 proniosomal gel and Terazol®3.

Formulation	SC1.5 proniosomal gel release samples after different time intervals (hour)								SC1.5	Terazol®3
	0.5	1	2	3	6	9	12	24		
Inhibition Zone (mm)	3.87 ± 0.52	5.92 ± 0.84	7.86 ± 0.3	9.42 ± 0.46	11.2 ± 0.43	12.92 ± 0.51	14.4 ± 0.36	15.2 ± 45	13.7 ± 0.23	9.6 ± .63

Values are expressed as mean ± SD, n=3

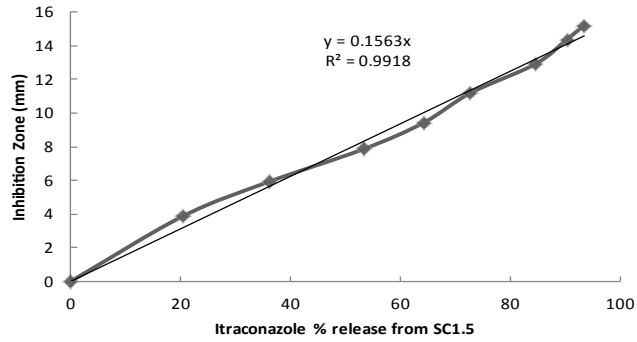

Figure 6: Correlation between dissolution samples of terconazole formulation SC1.5 in SVF and the inhibition zone.

Conclusion

Formulation of terconazole proniosomal gels to be hydrated and give terconazole niosomes was studied and evaluated. Variation between different formulations based mainly on ratio of cholesterol relative to the surfactant in addition to the surfactant type. In all formulations, the drug has been successfully entrapped. In most cases, increase cholesterol ratio had significant effects on the EE% and drug release profile from different formulations to give the highest EE % and optimized drug release profile in ratio of 1:1.5 cholesterol: span 60 use in formulation SC1.5. Incorporating the prepared proniosomes in carbopol gel enhanced its mucoadhesive properties. Microbiological evaluation of the optimized formulation, SC1.5 revealed that proniosomal gel formulations are promising formulations with good mucoadhesive properties, continuous drug release and efficient treatment of vaginitis.

References

1. Alsarra IA, Bosela AA, Ahmed SM, Mahrous GM (2005) Proniosomes as a drug carrier for transdermal delivery of ketorolac.Eur J Pharm Biopharm 59: 485-490.
2. Hunt C, Tsang S (1981) Tocopherol retards auto-oxidation and prolongs the shelf-life of liposomes. Int J Pharm 8: 101–110.
3. Wong M, Thompson TE (1982) Aggregation of dipalmitoylphosphatidylcholine vesicles.Biochemistry 21: 4133-4139.
4. Müller RH, Radtke M, Wissing SA (2002) Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations.Adv Drug Deliv Rev 54 Suppl 1: S131-155.
5. Hu C, Rhodes DG (1999) Proniosomes: a novel drug carrier preparation.Int J Pharm 185: 23-35.
6. Hanan ME, Omar S, Laila GM (2011) Novel sugar esters proniosomes for transdermal delivery of vinpocetine: Preclinical and clinical studies. Eur J Pharm Biopharm 77: 43–55.
7. Thomas L, Jose S, George SJ, Vidya V (2011) Provesicular niosomes gel: a novel absorption modulator for transdermal delivery. Int J Drug Dev Res 3: 58-69.
8. Nyririesy P, Weitz MV, Grody MH, Lorber B (2001) Chronic vulvovaginal candidiasis. Am Fam Physician 63:697–702.
9. Sobel JD (1988) Pathogenesis and epidemiology of vulvovaginal candidiasis. Ann N Y Acad Sci 544: 547-557.
10. Van Cutsem J (1991) The in vitro activity of terconazole against yeasts: its topical long-acting therapeutic efficacy in experimental vaginal candidiasis in rats.Am J Obstet Gynecol 165: 1200-1206.

11. Hire NN, Gudsoorkar VR, Bhise KS, Upasani CD, Nandgude TD et al. (2007) Microparticulate drug delivery system for topical administration of ITR. Asian J Pharmaceu 1:83–88.
12. Ghannoum MA, Rice LB (1999) Antifungal agents: mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance.Clin Microbiol Rev 12: 501-517.
13. Margareth RC, Raimar L, May A (2011) Simulated Biological Fluids with Possible Application in Dissolution Testing, Dissolution Technologies August: 15-28.
14. Yenkar P, Rahul M, Nawale R, Chavan R, Salunke T (2013) Bio Responsive In Situ Gel of Clindamycin for Vaginal Application. Research and Reviews: Journal of Pharmacy and Pharmaceutical Sciences 2: 26-32.
15. El-Gindy GA, El-Sayed AM, Mohamed AA, Mohamed SA, Abdel-Aal GM (2003) Mucoadhesive vaginal tablets of natamycin for vaginal candidiasis. Bull Pharm Sci Assiut University 26: 29-40.
16. Nitin BD, Badhan AC, Mashru RC (2009) A Novel itraconazole bioadhesive film for vaginal delivery: design, optimization, and physicodynamic characterization. AAPS PharmSciTech. 10: 951-959.
17. El Laithy HM, El-Shaboury KM (2002) The development of Cutina lipogels and gel microemulsion for topical administration of fluconazole.AAPS PharmSciTech 3: E35.
18. Huang L, Shu-Ping Y (2006) Method for preventing and/or treating vaginal and vulval infections. US Patent, US2006/0217443 A1.
19. Vora B, Khopade AJ, Jain NK (1998) Proniosome based transdermal delivery of levonorgestrel for effective contraception.J Control Release 54: 149-165.
20. Navin KD (2011) Preparation and characterization technique in niosomal vesicular systems-A review, Journal of Pharmaceutical and Biomedical Science 5:1-8
21. Balakrishnan P, Shanmugam S, Lee WS, Lee WM, Kim JO, et al. (2009) Formulation and in vitro assessment of minoxidil niosomes for enhanced skin delivery.Int J Pharm 377: 1-8.
22. Bernsdorff C, Wolff A, Winter R, Gratton E (1997) Effect of hydrostatic pressure on water penetration and rotational dynamics in phospholipids–cholesterol bilayers. Biophys J 72: 1264-1277.
23. Kirby C, Clarke J, Gregoriadis G (1980) Effect of the cholesterol content of small unilamellar liposomes on their stability in vivo and in vitro.Biochem J 186: 591-598.
24. Uchegbu IF, Vyas SP, Nonionic surfactant based vesicles (niosomes) in drug delivery. Int J Pharm 172: 33-70.
25. Vemuri S, Yu CD, deGroot JS, Roosdorp N, (1990) In vitro interaction of sized and unsized liposome vesicles with high density lipoproteins. Drug Dev Ind Pharm 16: 1579–1584.
26. Virtanen JA, Ruonala M, Auhkonen MV, Somerharju P (1995) Lateral organization of liquid-crystalline cholesterol–dimyristoylphosphatidylcholine bilayers. Evidence for domains with hexagonal and centered rectangular cholesterol superlattices. Biochemistry 34: 11568-11581.
27. El-Samaligy MS, Afifi NN, Mahmoud EA (2006) Increasing bioavailability of silymarin using a buccal liposomal delivery system: preparation and experimental design investigation.Int J Pharm 308: 140-148.
28. New RR (1990) Liposomes: A Practical Approach. Oxford University Press, Oxford.
29. Vinod KR, Santhosh V, Anbuazaghan S, David B, Padmasri A, et al. (2010a) Approaches for gastrotentive drug delivery systems. Int J Appl Bio Pharm Tech 1: 589-601.

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